

Abstract

P2X7 receptor is trimeric ligand-gated ion channel activated by extracellular ATP. This receptor is expressed in various tissues and cell types, such as glial cells, immune system cells or Schwann cells, different epithelial tissues and sperm cells, and is involved in many cellular physiological processes such as metabolism regulation, phagocytosis or apoptosis. The P2X7 subunit is composed of intracellular N- and C-termini, two transmembrane domains (TM1 and TM2) and a large extracellular ligand-binding domain. P2X7 has low sensitivity for its natural agonist, but prolonged or repeated applications lead to its sensitization and increase in current amplitude. Activation of P2X7 receptor is also known to induce uptake of large organic ions, such as fluorescent dye ethidium bromide. Receptor sensitization is accompanied by prolongation deactivation, mechanism of which is still unknown, that can be measured as current decay evoked by washout of agonist. This work explores the role of individual TM1 residues in deactivation kinetics of the receptor both in naïve and sensitized states. Electrophysiological whole-cell patch clamp method was used to record agonist-stimulated membrane current and its decay of wildtype P2X7 receptor (P2X7-WT) and TM1 alanine or leucine mutants. Mutations that were found to impair or change receptor deactivation kinetics were also tested for their ability to regulate ethidium bromide uptake function of receptor. In particular, Gly27Ala, Trp31Ala, Leu33Ala, Thr36Ala, and Val41Ala were observed to differ in their deactivation kinetics in naïve state compared to P2X7-WT, and all but one (Thr36Ala) differed also in sensitized state, that was also changed in Phe38Ala. Impairment in ethidium bromide uptake was observed in all of these mutations except Thr36Ala. A model of rat P2X7 revealed that residues Trp31, Leu33 and Val41A are important for TM1 and TM2 interaction and/or for interaction with lipids and lipophilic molecules in the membrane. This work indicates that numerous residues in the TM1 regulate P2X7 deactivation kinetics that determine dye uptake function of this receptor.

Keywords:

P2X7 receptor, extracellular ATP, purinergic signaling, receptor deactivation, transmembrane domain